PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 473/16, A61K 31/505 — A1

(11) International Publication Number: WO 98/16528

(43) International Publication Date: 23 April 1998 (23.04.98)

(21) International Application Number: PCT/US97/19472

(22) International Filing Date: 10 October 1997 (10.10.97)

(30) Priority Data:
60/028,306 11 October 1996 (11.10.96) US

(71) Applicants: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).

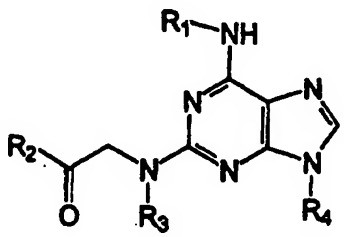
(72) Inventors: SCHULTZ, Peter; 4616 Rising Hill Court, Oakland, CA 94619 (US). RING, David, B.; 2375 Cowper Street, Palo Alto, CA 94301 (US). HARRISON, Stephen, D.; 1369 Francisco Street, Berkeley, CA 94702 (US). BRAY, Andrew, M.; 48 Western Road, Boronia, VIC 3155 (AU).

(74) Agents: FUJITA, Sharon, M. et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PURINE INHIBITORS OF GLYCOGEN SYNTHASE KINASE 3 (GSK3)



(1)

(57) Abstract

Compounds of formula (1) wherein R_1 is alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, substituted with 0-3 substituents selected from lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl-amino, and nitro; R_2 is hydroxy, amino, or lower alkoxy; R_3 is H, lower alkyl, lower acyl, lower alkoxy-acyl, or amino-acyl; R_4 is H or lower alkyl; and pharmaceutically acceptable salts and esters thereof; are effective inhibitors of GSK3.

PURINE INHIBITORS OF GLYCOGEN SYNTHASE KINASE 3 (GSK3)

Government License Rights

The U.S. Government has rights in this invention as provided for by the terms of contract No. DE-AC03-76SF00098 awarded by the Department of Energy (DOE) and by the terms of Postdoctoral Fellowships awarded by the National Science Foundation and the American Cancer Society.

Description

Field of the Invention

This invention relates generally to the field of medicinal chemistry, and specifically to compounds which inhibit the activity of glycogen synthase kinase 3 (GSK3).

Background of the Invention

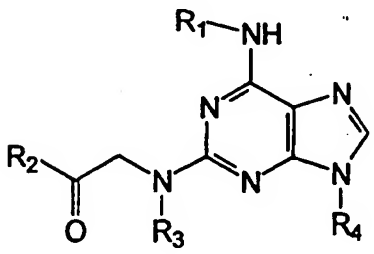
Glycogen synthase kinase 3 (GSK3) is a proline-directed serine/threonine kinase originally identified as an activity that phosphorylates glycogen synthase, as described in Woodgett, Trends Biochem Sci (1991) 16:177-81. GSK3 consists of two isoforms, α and β, and is constitutively active in resting cells, inhibiting glycogen synthase by direct phosphorylation. Upon insulin activation, GSK3 is inactivated, thereby allowing the activation of glycogen synthase and possibly other insulin-dependent events. Subsequently, it has been shown that GSK3 activity is inactivated by other growth factors or hormones, that, like insulin, signal through receptor tyrosine kinases (RTKs). Examples of such signaling molecules include IGF-1 and EGF as described in Saito *et al,* Biochem J (1994) 303:27-31; Welsh *et al,* Biochem J (1993) 294:625-29; and Cross *et al,* Biochem J (1994) 303:21-26. GSK3 has been shown to phosphorylate β-catenin as described in Peifer *et al,* Develop Biol (1994) 166:543-56.

Other activities of GSK3 in a biological context include GSK3's ability to phosphorylate tau protein *in vitro* as described in Mandelkow and Mandelkow, Trends in Biochem Sci (1993) 18:480-83; Mulot *et al,* FEBS Lett (1994) 349:359-64; and Lovestone *et al,* Curr Biol (1995) 4:1077-86; and in tissue culture cells as described in

Detailed Description

Definitions

The term "compound of formula 1" refers to compounds having the general structure:



wherein

R_1 is alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, substituted with 0-3 substituents selected from lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl-amino, and nitro;

R_2 is hydroxy, amino, or lower alkoxy;

R_3 is H, lower alkyl, lower acyl, lower alkoxy-acyl, or amino-acyl;

R_4 is H or lower alkyl;

and pharmaceutically acceptable salts and esters thereof.

The term "alkyl" as used herein refers to saturated hydrocarbon radicals containing from 1 to 12 carbon atoms, inclusive. Alkyl radicals may be straight, branched, or cyclic. Exemplary alkyl radicals include n-pentyl, n-hexyl, n-octyl, n-dodecyl, 2-dodecyl, 3,5-diethylcyclohexyl, duryl, and the like. The term "lower alkyl" as used herein refers to straight, branched, and cyclic chain hydrocarbon radicals having from 1 to 8 carbon atoms, such as methyl, ethyl, propyl, isopropyl, n-butyl, s-butyl, t-butyl, n-pentyl, n-hexyl, cyclopentyl, cyclohexyl, 2-methylcyclopentyl, and the like. "Alkoxy" refers to radicals of the formula -OR, where R is alkyl as defined above: "lower alkoxy" refers to alkoxy radicals wherein R is lower alkyl. "Hydroxy-lower alkyl" refers to radicals of the formula HO-R-, where R is lower alkylene of 1 to 8 carbons, and may be straight, branched, or cyclic. "Hydroxy-lower alkoxy" refers to radicals of the formula HO-R-O-, where R is lower alkylene of 1 to 8 carbons, and may be straight, branched, or cyclic. "Lower alkoxy-lower alkyl" refers to groups of the formula R_aO-R_b-, where R_a and R_b are each independently lower alkyl.

The term "halo" refers to a halogen radical, such as F, Cl, Br, or I.

The term "treatment" as used herein refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder in a subject who is free therefrom. Thus, for example, treatment of non-insulin dependent diabetes melitis (NIDDM) in a patient may be reduction in the serum levels of glucose. Treatment of Alzheimer's disease may be halting or retarding the progression of the disease (e.g., as measured by a reduction in the rate of dementia).

The term "glycogen synthase kinase 3" or "GSK3" as used herein refers to a protein originally identified by its phosphorylation of glycogen synthase as described in Woodgett *et al*, Trends Biochem Sci (1991) 16:177-81.

The term "biological condition mediated by GSK3 activity" as used herein refers to any biological or medical condition or disorder in which effective GSK3 activity is identified, whether at normal or abnormal levels. The condition or disorder may be caused by the GSK3 activity or may simply be characterized by GSK3 activity. That the condition is mediated by GSK3 activity means that some aspect of the condition can be traced to the GSK3 activity.. It is expected that by the method of the invention, inhibiting the GSK3 activity will then prevent, ameliorate or treat the condition so characterized.

The term "CREB peptide" as used herein refers to a sequence within the CREB DNA-binding protein as described in Wang *et al*, Anal. Biochem (1994) 220:397-402.

General Methods and Detailed Description

The present invention provides for the inhibition of GSK3 activity, which includes, for example, inhibition of its kinase activity. By inhibiting GSK3 kinase activity, other activities downstream of GSK3 kinase activity are inhibited. For example, inhibition of the GSK3 kinase activity can result in the activation of glycogen synthase, because normally GSK3 acts constitutively in cells to inactivate glycogen synthase by direct phosphorylation. When GSK3 kinase activity is inhibited, glycogen synthase may activate leading to a cascade of events. GSK3 is also known to act as a kinase in a variety of other contexts, including but not limited to, for example, phosphorylation of c-jun, β-catenin, and tau protein. It is understood that inhibition of GSK3 kinase activity

temperature. The resulting compound (with $R_2 = NH_2$) may be modified to provide other R_2 substitutions by standard techniques (esterification, etc.).

In presently preferred compounds of the invention, R_1 is either a lipophilic alkyl (such as 3-methylbutyl, pentyl, hexyl, cyclohexyl, and the like, preferably 3-methylbutyl) or a substituted benzyl or pyridylmethyl radical, especially 4-fluorobenzyl, 3-pyridylmethyl, 4-pyridylmethyl, 4-trifluoromethylbenzyl, 4-methoxybenzyl, or 4-chlorobenzyl. R_2 is preferably NH_2, and R_4 is preferably lower alkyl, particularly methyl. R_3 is preferably H or acyl, optionally substituted with NH_2 or CH_3O, especially propionyl, 2-aminoacetyl, 2-methoxyacetyl, 3-methylbutyryl, 3-methoxypropionyl, butyryl, or 3-aminopropionyl.

Compounds of the invention are assayed for activity by standard techniques, preferably employing the GSK3 assay described in the examples below. The methods include methods to assay for GSK3 kinase activity in an *in vitro* or cell-based assay, a method to assay for inhibitors of binding to GSK3, and an *in vivo Drosophila* eye screening assay.

General aspects of the kinase activity assays are conducted as described in U.S. Patent No. 4,568,649; EP 154,734; and JP 84/52452, incorporated herein by reference, which describe kinase activity assays conducted for kinases other than GSK3. It is believed that GSK3 isoforms α and β phosphorylate serine and threonine residues in the amino acid context serine-proline (SP) or threonine-proline (TP), as well as at the N-terminal serine in the motif SXXXS, provided that the C-terminal serine in this sequence is prephosphorylated, as described in Wang *et al*, Anal Biochem (1994) 220:397-402 and Roach, J Biol Chem (1991) 266:14139-42.

Two of the methods for identifying specific inhibitors of GSK3 are an *in vitro* kinase assay and a cell-based kinase assay. Both kinase assays are performed similarly, with the distinction between them that the *in vitro* assay screens inhibitors that will act on the polypeptide GSK3, and the cell-based assay screens in addition for inhibitors that can act within the cell at any step in the process of expression of GSK3. Thus, the cell-based assay can screen for, for example, those inhibitors that act during transcription of GSK3 or that can act during intracellular post-transcriptional events in the process of making mature GSK3.

light and the measure of the amount of light emitted will be a measure of the activity of GSK3 in the assay. Low activity of GSK3 observed in the presence of a candidate inhibitor, as compared to the activity of GSK3 in the absence of the inhibitor, may indicate that the inhibitor is functional and can inhibit GSK3 kinase activity. In any case, an equal amount of streptavidin should be loaded into each well or should be affixed to the agarose beads, and an equal amount of the beads added to each assay.

The cell-based assay includes in addition, a cell that can express GSK3, such as for example a cell transformed with the gene encoding GSK3, including regulatory control sequences for the expression of the gene. For conducting the cell-based assay, the cell capable of expressing GSK3 is incubated in the presence of a compound of the invention, the cell is lysed and the GSK3 is immunoprecipitated or otherwise purified, and the purified GSK3 is placed in contact with a peptide substrate, and radiolabeled phosphate-ATP. The amount of phosphorylation of the substrate is an indication of the degree of inhibition accomplished by the compound of the invention. During the cell-based assay, inhibition of GSK3 activity may occur either by inhibiting the expression of GSK3 or by inhibition of GSK3's protein kinase activity, both of which will be indicated by phosphorylation (or lack thereof) of the substrate peptide in the cell-based assay. However, one can determine which aspect is inhibited by assaying inhibition of GSK3 *in vitro* as described above.

An alternative assay that can be used to screen *in vivo* for inhibitors of GSK3 kinase activity is a *Drosophila* eye screen for inhibitors. The fly eye screen detects inhibitory activity by expressing GSK3 in *Drosophila* under the control of an eye-specific promoter. The eye specific promoter can be any promoter specific to expression of proteins in eye tissue, including but not limited to, for example, GMR as described in Hay *et al,* Development (1994) 120:2121-29, and the sevenless promoter, as described in Bowtell *et al,* Genes and Development (1988) 2:620-34. The screening assay for inhibitors of GSK3 activity is then conducted by feeding the flies food containing a compound of the invention. If the inhibitor is functional, the eye morphology reverts from mutant to wildtype. The expression of GSK3 under the control of the eye specific promoter leads to developmental defects which result in obvious aberrations in the external morphology of the external eye tissue. The mutant morphology that results in

acetate, gelatin, collagen, Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like. Cream or ointment bases useful in formulation include lanolin, Silvadene® (Marion), Aquaphor® (Duke Laboratories), and the like. Other topical formulations include aerosols, bandages, sustained-release patches, and the like. Alternatively, one may incorporate or encapsulate the compound in a suitable polymer matrix or membrane, thus providing a sustained-release delivery device suitable for implantation near the site to be treated locally. Other devices include indwelling catheters and devices such as the Alzet® minipump. Further, one may provide the compound in solid form, especially as a lyophilized powder. Lyophilized formulations typically contain stabilizing and bulking agents, for example human serum albumin, sucrose, mannitol, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co.).

Examples

The examples presented below are provided as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

Example 1

(Preparation of Compounds)

Compounds of the invention were prepared following the method disclosed in T.C. Norman *et al.*, J. Am. Chem. Soc. (1996) 118:7430-31.

(A) 2-Amino-6-chloropurine was treated with NaH (1.1 eq) and CH_3I (1 eq) in DMF. The product was treated with trifluoroacetic anhydride (3 eq) in CH_2Cl_2, then alkylated with *t*-butyl α-iodoacetate (2 eq) and NaH (1.1 eq) in DMF. The reaction was quenched with K_2CO_3 in MeOH. The product was then treated with trifluoroacetic acid (TFA) and 1,4-dimethoxybenzene, followed by PyBroP (1 eq), *p*-nitrophenol (1 eq), and DIEA (3 eq) in CH_2Cl_2, and coupled to Rink-derivatized polyethylene crowns

(B) Proceeding as in part (A) above, the following compounds were made:

Compound ID	R_1	R_2	R_3	R_4
21172	F_3C-ϕ-CH_2- (*)	NH_2	H	CH_3
21232	F_3C-ϕ-CH_2-	NH_2	NH_2CH_2CO-	CH_3
21220	F_3C-ϕ-CH_2-	NH_2	CH_3CH_2CO-	CH_3
20957	2-pyridylmethyl	NH_2	H	CH_3
20981	2-pyridylmethyl	NH_2	$(CH_3)_2CHCH_2CO$	CH_3
21005	2-pyridylmethyl	NH_2	CH_3CH_2CO-	CH_3
21132	$(CH_3)_2CHCH_2CH_2$	NH_2	CH_3OCH_2CO	CH_3
21131	4-F-ϕ-CH_2-	NH_2	CH_3OCH_2CO	CH_3
21196	F_3C-ϕ-CH_2-	NH_2	$(CH_3)_2CHCH_2CO$	CH_3
21095	4-F-ϕ-CH_2-	NH_2	H	CH_3
21100	4-CH_3O-ϕ-CH_2	NH_2	H	CH_3
20951	cyclohexyl	NH_2	H	CH_3
20956	4-pyridylmethyl	NH_2	H	CH_3
21004	4-pyridylmethyl	NH_2	$CH_3(CH_2)_2CO$	CH_3
21075	4-Cl-ϕ-CH_2	NH_2	$CH_3(CH_2)_2CO$	CH_3
21156	$(CH_3)_2CH(CH_2)_2$	NH_2	$NH_2(CH_2)_2CO$	CH_3

(*)ϕ = phenyl

Example 2

(Assay)

(A) A GSK3β gene was created in which a haemagluttinin (HA) epitope was fused to the N-terminal end of the GSK3β open reading frame in plasmid vector pCG, a pEVRF derivative, described in Giese *et al.* Genes & Development (1995) 9:995-1008, and in Matthias *et al.*, Nucleic Acids Res. (1989) 17:6418. pCG has a modified polylinker, and directs expression in mammalian cells from the human cytomegalovirus promoter/enhancer region. The resulting plasmid is pCG-HA-GSK3β. pCG-HA-GSK3β was transiently transfected into COS cells on 10 cm tissue culture plates using DEAE

phosphorylated. This experiment demonstrates the specificity of the peptide as a GSK3 substrate.

(B) The compounds prepared in Example 1 above were assayed for activity as follows:

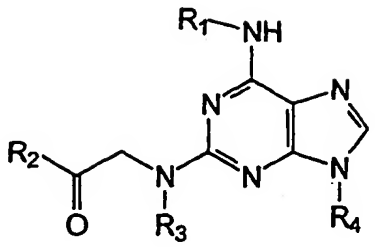
A 96-well round bottom plate was blocked by incubation with 400 µl/well 1% BSA/PBS for 60 min at room temperature. The blocking reagent was then aspirated. An enzyme/substrate buffer was prepared (1.225 ml 1M Tris-HCl, pH 7.5, 0.41 ml 1M $MgCl_2$, 41 µl DTT, 250 µl 500 µg/ml GSK-3β, 9.5 µl 5 mg/ml biotin-phosphopeptide, 33.1 ml 1% BSA/PBS), and 300 µl enzyme/substrate/buffer was added to each well.

Varying concentrations of each compound were added to individual wells, or staurosporine in DMSO (final concentrations of staurosporine 100 nM or 20 nM). Next 50 µl/well ATP mixture (3.85 µl 10 mM cold ATP, 8 µl hot ATP, 5.5 ml H_2O) was added, and the reaction allowed to proceed for 180 min at room temperature.

Three streptavidin-coated Labsystems Combiplate 8 plates were blocked with 1% BSA/PBS, 300 µl/well, for ≥60 min at room temperature, and the blocking reagent aspirated. Stopping reagent (50 µM ATP, 20 mM EDTA) (100 µl/well) was added to the streptavidin-coated plates, and 100 µl of the enzyme reaction mixture transferred to the streptavidin-coated plates in triplicate. The plates were incubated at room temperature for 60 min, and washed 5X with PBS using a Corning plate washer. Finally, Microscint-20 scintillation fluid (200 µl/well) was added to the plates, the plates sealed, and after incubating for 30 min, counted on a TopCount counter. The results were as follows:

What is claimed:

1. A compound of formula 1:



wherein

R_1 is alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, substituted with 0-3 substituents selected from lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl-amino, and nitro;

R_2 is hydroxy, amino, or lower alkoxy;

R_3 is H, lower alkyl, lower acyl, lower alkoxy-acyl, or amino-acyl;

R_4 is H or lower alkyl;

and pharmaceutically acceptable salts and esters thereof.

2. The compound of claim 1, wherein R_4 is methyl.

3. The compound of claim 2, wherein R_2 is amino.

4. The compound of claim 3, wherein R_1 is pyridylmethyl.

5. The compound of claim 4, wherein R_3 is propanoyl.

6. The compound of claim 4, wherein R_3 is 3-methylbutanoyl.

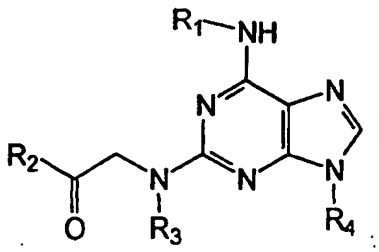
7. The compound of claim 4, wherein R_3 is butanoyl.

8. The compound of claim 4, wherein R_3 is H.

23. The compound of claim 3, wherein R_1 is 4-chlorobenzyl, and R_3 is butanoyl.

24. A pharmaceutical composition, comprising:

a compound of formula 1:



wherein

R_1 is alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, substituted with 0-3 substituents selected from lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl-amino, and nitro;

R_2 is hydroxy, amino, or lower alkoxy;

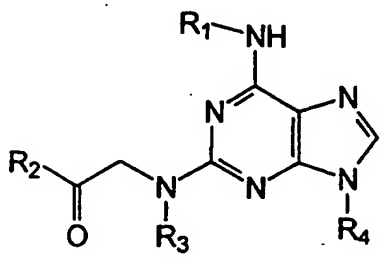
R_3 is H, lower alkyl, lower acyl, lower alkoxy-acyl, or amino-acyl;

R_4 is H or lower alkyl;

or a pharmaceutically acceptable salts and esters thereof; and

a pharmaceutically acceptable excipient.

26. A method for treating a disorder mediated by GSK3, comprising: providing an effective amount of a compound of formula 1:



wherein

R_1 is alkyl, cycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl, substituted with 0-3 substituents selected from lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl-amino, and nitro;

R_2 is hydroxy, amino, or lower alkoxy;

R_3 is H, lower alkyl, lower acyl, lower alkoxy-acyl, or amino-acyl;

R_4 is H or lower alkyl;

or a pharmaceutically acceptable salt or ester thereof; and

administering said composition to a subject having a disorder mediated by GSK3.

27. The method of claim 26, wherein said disorder is selected from the group consisting of diabetes and Alzheimer's disease.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 616 032 A (MITSUBISHI CHEM IND) 21 September 1994 see claim 1	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[redacted] Application No
PCT/US 97/19472

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9716452 A	09-05-97	AU 7296896 A	22-05-97
WO 9741854 A	13-11-97	NONE	
WO 9722360 A	26-06-97	AU 1200497 A	14-07-97
EP 0616032 A	21-09-94	JP 6253835 A	13-09-94
		JP 6329551 A	29-11-94
		CA 2116460 A	03-09-94